The tailocin tale: peeling off phage tails

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Abstract

Bacteria produce a variety of particles resembling phage tails that are functional without an associated phage head. Acquired from diverse bacteriophage sources, these stand-alone units were sculpted to serve different ecological roles. Such tailocins mediate antagonism between related bacteria as well as interactions with eukaryotic cells.

Killer tails

In bacteria, multiple strategies to combat microbial competitors have evolved, including the release of inhibitory substances, either derived from secondary metabolism or (poly)peptide synthesis. Whereas the antibiotic activity of secondary metabolites tends to affect mostly unrelated bacteria, bacteriotoxic peptides and proteins frequently target close relatives, some even affecting strains of the producer's own species. Historically, mediators of such protein-based activity were collectively denoted bacteriocins. In recent years, similar features have been discovered in a variety of so-called polymorphic toxins [1], including certain substrates of the type VI secretion machinery.

'Tailocins' are multi-protein particles morphologically similar to phage tails [2] and bacteriocin activity has been demonstrated for a subset of them. In the opportunistic human pathogen *Pseudomonas aeruginosa*, tailocins display a flexible (F) or rigid (R) appearance. These particles, denoted F- and R-type pyocins, respectively, have served as models for phage-like bacteriocins [3]. R-type tailocins also occur in plant-associated pseudomonad species [3, 4]. Morphologically similar bacteriocins have been identified in several other γ-proteobacterial genera, representing species with different lifestyles, such as carotovoricin of the phytopathogen *Pectobacterium carotovorum* causing soft rot, xenorhabdicin of the entomopathogenic nematode symbiont *Xenorhabdus nematophila*, and maltocin of the opportunistic pathogen *Stenotrophomonas maltophilia* [5, 6]. Bactericidal tailocins are not confined to Gram-negative bacteria, as their general morphology is also conserved in a bacteriocin produced by the Gram-positive pathogen *Clostridium difficile* [7]. Elucidation of the tube and sheath structure of the R pyocin contractile tail revealed both architectural similarities and differences with the nanotubes of phage tails and of the type VI secretion machinery (Figure 1) [8].

Although tailocins bear striking morphological similarity to phage tails, their genetic makeup indicates that they should not be considered degenerate prophages. Moreover, they have evolved extensively to fulfill diverse ecological roles, as explained below.

Headless but still functional

The F- and R-type tailocin gene clusters display striking synteny with the genomic regions for tail assembly of phages belonging to the families *Siphoviridae* (e.g. HK022) and *Myoviridae* (e.g. P2), respectively (http://viralzone.expasy.org) [9]. However, in line with their headless tail morphology, the corresponding genes for phage head assembly and DNA packaging are lacking. On the other hand, the gene clusters encompass cognate regulatory genes and dedicated lysis cassettes for release of the particles. This indicates that these tailocins are expressed from well-organized functional units. To assess the tailocin-encoding potential of such genomic regions, careful delineation and comparative analyses of synteny and gene content are required. Frequently, intact prophages and tailocin clusters with highly similar tail regions are present in a particular strain, sometimes located adjacently.

Different trails of tail domestication

Genomic tailocin clusters of the R- and F-type are abundantly present in the genomes of *P. aeruginosa* strains, either individually or as R-to-F fused regions similar to the pyocin R2-F2 gene organization in *P. aeruginosa* PAO1 [3]. Two additional tailocins resembling different *Myoviridae* (pro)phages have been identified in *Pseudomonas fluorescens* and in *Pseudomonas syringae* [3, 4]. Phylogenetic analysis of structural components shared between phages and the abundant R-type tailocins reveals a distinct clustering with different phage genera, as illustrated for their sheath proteins in Figure 2A. Whereas the R2-type pyocin appears to be related to a pseudomonad phage (PS17), a probable ancestry shared with non-pseudomonad phages of different genera is inferred for other pseudomonad tailocins (e.g. *Vibrio parahaemolyticus* phage VP882, *Hapunalikevirus* genus; *Shigella flexneri* phage SfV, *Mulikevirus* genus). This strongly argues against a single common origin and subsequent diversification. Despite the presence of a similar fold in sheath proteins of

pyocin R2 and the type VI secretion tubule, sequence homology between the respective protomers is essentially lacking [8].

In addition to individual occurrence of one of the four tailocin types (F-type and three R-subtypes), pseudomonad genomes exhibit a remarkable variety of tailocin combinations with up to three different units, recruited to two genomic hotspots (Figure 2B). The presence of a single set of regulatory and lysis genes flanking such mosaic tail regions strongly suggests that tailocin pairs or triplets are released in a coordinated fashion, as demonstrated for the pyocin R2-F2 locus of *P. aeruginosa* PAO1 [3]. In some strains, individual or paired tailocin clusters are co-integrated with an apparently intact prophage, with its own regulatory and lysis genes and not necessarily carrying a tail region of the same type.

Teaching tails new tricks

In contrast to the conservation of most structural proteins of *Pseudomonas* F- and R-type tailocins, tail fiber proteins (and cognate chaperones) only display borderline protein sequence identity [3]. Acting as key determinants of target selectivity, this points to a potentially tremendous reservoir of different specificities. Previously, it was demonstrated that R pyocin target spectra, mediated by lipopolysaccharide binding, can be altered by the construction of chimeric tail fibers, via exchange of tail fiber fragments from tailocins, as well as from phages [10]. Moreover, functional pyocins were generated by introduction of tail fiber genes from prophage regions, present in pathogens of interest. Solely based on genome information, this strategy was applied successfully to produce artificial R pyocins targeting Shiga toxin-producing *Escherichia coli*, and more recently, for diffocins killing *C. difficile* [7]. Potent activity, a relatively high *in vivo* stability, and the absence of genetic material may make such engineered tailocins an attractive platform to combat pathogens for which efficient treatment or prophylaxis is lacking.

Tails tailored for protein translocation

Bactericidal tailocins represent proton motive force-dissipating devices that perforate the cell envelope of target bacteria [8]. In addition, syringe-like assemblies composed of similar phage tail-

derived building blocks are proposed to enable certain bacteria to inject toxic proteins into eukaryotic cells. Although lacking bacteriocin activity, these tubular devices display morphological similarity to R-type tailocins and sequence homology between some common components such as the sheath and tube proteins, of which multiple copies can be present. Sheath-based phylogeny illustrates their clustering, separated from the bacteriocin branch (Figure 2A). An insecticidal cargo can be delivered into insect larvae through Afp (antifeeding prophage) by *Serratia entomophila* and through PVC (*Photorhabdus* virulence cassette) by *Photorhabdus* species [2]. The wide distribution of Afp/PVC-like gene clusters in prokaryotes prompted Sarris *et al.* [11] to collectively designate such particles as a distinct type of secretion system, phage-like protein-translocation structures (PLTSs). A specialized function has been assigned to a PLTS-like entity in *Pseudoalteromonas luteoviolacea* [12]. This surface-colonizing bacterium releases ordered arrays of contractile tailocins, designated MAC (metamorphosis-associated contractile structure), that induce metamorphosis in settling larvae of the benthic marine tubeworm *Hydroides elegans* upon contact. In addition to MAC, *P. luteoviolacea* also produces a typical R-type tailocin, quite similar to maltocin [12]. No function has yet been ascribed to the PLTS-like structures, originally designated rhapidosomes, observed inside the gliding marine bacterium *Saprospira grandis* preying on other bacteria [11]. Similar PLTS-like gene clusters of unknown function have been revealed by genomic sequencing in other members of the *Bacteroidetes*, living as symbionts of insects (e.g. *Cardinium hertigii*) or amoebae (e.g. *Amoebophilus asiaticus*). Analogous to type VI secretion systems but unlike the bactericidal tailocins, PLTS clusters harbor an AAA+ ATPase [11]. This protein is required for Afp assembly. The absence of a lysis cassette, proposed as a hallmark of PLTS systems by Sarris *et al.* [11], does however not apply to the Afp cluster, which encompasses such a module encoding putative holin/endolysin/spanin genes that potentially mediate tailocin release from a producer cell.

Concluding remarks

Tailocins illustrate the daedalian capacity of bacteria to accommodate exogenous genetic elements and domesticate them for their own benefit. The stinging device used by tailed (bacterio)phages against bacteria has been cunningly converted into tools to manipulate eukaryotic cells and into

precision weapons for interbacterial warfare. The further elucidation of tailocin structures in molecular detail and a profound insight in their assembly and function as molecular machines will likely reveal additional ecological roles associated with these particles.

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Figure legends

Figure 1. Structure of a bactericidal tailocin. (A) The structure consists of a rigid tube (orange subunits), contractible sheath (blue subunits), LPS-targeting tail fibers (red) attached to the baseplate (grey), and spike (black) connected via the baseplate hub (pale yellow) to the central tube. (B) Tail-tube architecture of pyocin R2 in extended conformation (EMDB-6270, PDB 3J9Q) with top view of a transversal section showing a hexameric disc (marked with a box in panel A). Two sheath protomers (cyan and blue) and two tube protomers (yellow and orange) are shown in surface representation; the other subunits (cartoon) are shown in grey (sheath) and white (tube).

Figure 2. Diversity of phage-tail-like particles. (A) Maximum-likelihood tree inferred from a multiple sequence alignment of sheath proteins of bacteriocins (upper box) and of structurally related particles (lower box) with homologues encoded by selected *Myoviridae* phages (names in italic/blue font) infecting different hosts (genera specified between brackets), with the *Bacillus cereus* phage BCD7 protein taken as root. The proteins of three R-type tailocins characterized in pseudomonads are marked with a glowing rounded square: syringacin of *P. syringae* (purple), pyocin R2 of *P. aeruginosa* (red), and 'fluorescin' of *P. fluorescens* (orange). The Afp and PVC gene clusters each encode three homologues. The *Cardinium hertigii* protein is included as a non-functionally characterized PLTS representative, together with the sheath protein MacS from *Pseudoalteromonas luteoviolacea* and the rhapidosome subunit from *Saprospira grandis*. The scale bar represents 0.5 substitutions per site. Bootstrap values are shown as percentages (1000 replicates). (B)

Representative tailocin genomic regions in pseudomonads located between *trpE* and *trpG* (grey arrows) or between *mutS* and *cinA* (white arrows). Tailocin gene clusters (represented by rounded rectangles) occur individually, or as pairs or triplets of four different (sub)types: three *Myoviridae* subtypes (colored according to sheath protein phylogeny in panel A) and a F-type with similarity to *Siphoviridae* phage tails (unrelated to the *Myoviridae*, pale blue). In some strains, tailocins are combined with an intact prophage carrying a tailocin-syntenic region of a particular (sub)type. The hexagon-oval combinations represent head-tail regions of such prophages. Gene clusters and flanking genes are not drawn to scale.

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